HALOGENATION OF COMPLEX ORGANIC COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Divisional of U.S. application Ser. No. 16/516,102, filed Jul. 18, 2019, which claims the priority benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/700,152, filed Jul. 18, 2018, the disclosure of which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under CA047135, CA070375 and R01 086374 awarded by the National Institutes of Health, and CHE-1205646 awarded by the National Science Foundation. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file. The name of the text file containing the Sequence Listing is "53295A_Seqlisting.txt", which was created on Jul. 18, 2019 and is 84,638 bytes in size. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.

FIELD

[0004] The disclosure relates generally to the field of complex chemistry and more particularly to biocatalysis of compound halogenation.

BACKGROUND

[0005] The prevalence of halogenated natural products has led to significant advances in understanding various classes of halogenases involved in secondary metabolism. Most halogenases characterized thus far can be placed into three classes: haloperoxidases (heme-containing and vanadium-containing), non-heme Fe(II)α-ketoglutarate-dependent, and flavin-dependent enzymes. Haloperoxidases are generally nonselective and perform halogenation through a mechanism utilizing freely diffusing hypohalous acid. By contrast, Fe(II)α-ketoglutarate-dependent halogenases proceed through a radical mechanism, typically halogenating aliphatic, unactivated carbons.[1] Flavin-dependent halogenases (FDHs) also proceed through a hypohalous acid intermediate, with the reactive reagent captured by a lysine residue that appears to control the regioselectivity of halogenation on aromatic substrates.[2,3] The FDH-derived hypohalous acid is generated through a reaction between the flavin C4a-peroxide adduct and the bound chloride ion. FDHs are thought to proceed through an electrophilic aromatic substitution (EAS) where the catalytic lysine residue provides the chloramine halogenating agent and a catalytic glutamate facilitates the reaction by deprotonating the positively charged intermediate generated during catalysis.[2]

[0006] The majority of previously characterized FDHs are of bacterial origin, with relatively few reported from eukaryotes,[4-12] and fewer still characterized biochemically.[4-6,10-12] The bacterial FDHs have been found to catalyze reactions on both free,[2,13-15] and carrier-protein-bound substrates,[16,17] including precursor amino acids in natural product biosynthesis. The well-characterized eukaryotic FDHs Rdc24 and ChlA6 catalyze late-stage C—H functionalization reactions in the biosynthesis of halogenated metabolites. However, structural data for these two enzymes have not been reported, and it has remained unclear how they control site-selective halogenation on large, structurally complex substrates.

[0007] Malbrancheamide (compound 1) is a complex halogenated indole alkaloid produced by the terrestrial fungus *Malbranchea aurantiaca* RRC181318 and the marine sponge-derived fungus *Malbranchea graminicola* 086937A.[19] The discovery of malbrancheamide was enabled by a search for calmodulin antagonists, and several studies have characterized its significant vasorelaxant effect.[20,21] Along with malbrancheamide, a close structural relative, spiromalbramide, was isolated from *M. graminicola*.[19] The two strains are highly related, with 99% sequence identity overall, and their biosynthetic pathways for malbrancheamide are proposed to be identical (Scheme 1). Malbrancheamide (compound 1) belongs to a family of prenylated indole alkaloids formed through peptide coupling by a nonribosomal peptide synthetase (NRPS), addition of an isoprene unit by a prenyltransferase, and a proposed [4+2] Diels-Alder cycloaddition to form the characteristic bicyclo[2.2.2]diazaoctane ring of premalbrancheamide (compound 2) (Scheme 1).[22-26] Premalbrancheamide is then proposed to be dichlorinated through an iterative mechanism, but whether this halogenation is performed by one or two halogenases remained to be determined.[25] The chlorination of the indole ring differentiates this molecule from the rest of its class and significantly contributes to its biological activity.[21]

[0008] In earlier efforts to elucidate the malbrancheamide biosynthetic pathway, precursor incorporation studies were performed in *M. aurantiaca*. This led to the conclusion that premalbrancheamide (compound 2) is indeed incorporated into the monochlorinated malbrancheamide B (compound 3) and that both compounds are natural metabolites of *M. aurantiaca*.[25] Previously, it had been proposed that there is a site-selective chlorination of the C9 position prior to functionalization of C8 for production of malbrancheamide (compound 1).[25] The isolation of both C8 (isomalbrancheamide B (compound 4)) and C9 (malbrancheamide B (compound 3)) monohalogenated metabolites from *M. aurantiaca*[20] and from *M. graminicola*[19], however, conflicted with the proposed C9 selectivity hypothesis.

[0009] The ability to selectively halogenate C—H bonds in highly complex molecules through synthetic methods has posed a formidable challenge due to the abundance of chemically equivalent C—H bonds, and the inability to overcome inherent steric or electronic bias for reactivity.[31,32] The large number of biologically active natural products that undergo late-stage functionalization by tailoring enzymes provides a unique opportunity to leverage the power of halogenating enzymes to perform difficult chemical transformations.

[0010] In view of the foregoing observations, a need continues to exist in the art for catalysts that modify complex compounds, for example the controlled halogenation of complex compounds such as indole alkaloids.